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Variations of β-carotene retention in a staple produced from yellow fleshed cassava roots through different drying methods

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ABSTRACT

Background: New yellow cassava fresh roots biofortified with β-carotene are becoming popular in Sub-Saharan Africa in the making of traditional products for households. However, β- carotene is susceptible to rapid depletion during processing. Therefore, we investigated the impact of using different drying methods in processing these fresh Cassava roots.

Methods: We processed four newly released yellow fleshed cassava varieties (01/1371, 07/593, 07/539, and 07/0220) into fermented cassava chips, flour, and corresponding dough with sun, oven, and flash drying methods respectively. The β-carotene contents were analyzed using High Performance Liquid Chromatography (HPLC). Percentage true retention (%TR) was computed.

Results: The results demonstrated that %TR in chips (13.7%), flour (11.7%), and dough (5.48%) from sun drying method had the highest level of β-carotene retention compared to oven (11.3%, 7.30%, and 3.47%) and flash (8.8%, 3.33%, and 1.24%) drying methods. The duration, intensity of heat used for drying, and variety had a significant effect on β-carotene retention. Variety 07/0220 with the highest β-carotene concentration (7.81±0.13μ/g) in the fresh roots did not necessarily have the highest concentration after processing. These results suggest that β-carotene retention is not only variety specific but also depends on the method of processing.

Conclusions: These results will support ongoing breeding efforts aimed at increasing pro-vitamin A content in Cassava. The information may also be significant to solutions considering fermented cassava flour and dough in Vitamin A deficient populations.

Keywords: Cassava, drying, fermented flour, carotenoids, retention, lafun

BACKGROUND

Cassava (*Manihot esculenta* Crantz) is a root crop widely grown in countries of tropical Africa. Its derived food products are a major staple in several countries of sub-Saharan Africa (SSA), being a major contribution to the daily energy intake of both urban and rural dwellers compared to other staples [1-3]. In recent years, successful biofortification efforts around the world have resulted in the release of cassava varieties rich in pro-vitamin A (pVA) in Cassava growing countries of Africa [4, 5]. These new varieties, besides contributing to the energy intake of consumers, are expected to be a vehicle of conveying pVA to vitamin A deficient (VAD) populations [6]. The fresh biofortified cassava roots are also widely referred to as yellow cassava.

Raw Cassava is bulky, with a moisture content of about 70%, making transportation to urban markets difficult and expensive. Fresh roots are highly perishable and cannot be stored for more than a few days after harvest [7, 8]. Cassava is often processed into products easier to store to avoid postharvest loss [9].

One of these products is fermented cassava flour, which is usually cooked into a stiff dough. The dough is prepared by vigorously stirring the flour in boiling water and is usually eaten with vegetable soup, stew, or sauce [10]. Fermented cassava flour is one of the desirable products from yellow cassava [11]. This local flour (and its dough) has varying names depending on the locality in different SSA countries and is referred to as *lafun, amala-lafun, makopa, ugali, chigan lafun,* and *Oka.* In southwestern Nigeria, this popular product is called *lafun*.

A major advantage of this fermented flour is the extended shelf life, making it easier and cheaper to transport, market, and—most importantly—providing long-term food security. In most populations of SSA where food security is still an unreached goal, this product has found popularity in households since it can provide relief during times of food shortage.

The development of Cassava varieties with increased levels of pro-vitamin A presents a further advantage for these products to not only solve the problems of hunger but also complement as a source of vitamin A intake. While the traditional method of processing varies with locality, the basic principles of fermenting the roots for a few days and drying before the conversion to flour is consistent. The flour is made into dough before consumption. Drying of the fermented roots is a key operation in the production of fermented cassava flour. Besides sun drying –which is the oldest and the most common method of drying cassava products—new methods such as solar, cabinet, oven, and flash drying are now being applied to reduce moisture content during processing [12, 13].

As reviewed by De Moura et al. [14], different methods of drying can have a significant impact in terms of retention of carotenoids in biofortified root crops like cassava and sweet potato. Thakkar et al [15] established that factors such as high temperatures and long term exposure could severely affect retention of carotenoids during Cassava processing. There is some information on the effect of processing methods on the physical, microbial, and nutritional qualities of fermented dried flour and dough from white varieties [15, 16, 17], especially the effects of drying methods on their physiochemical properties [18, 19].

However, there is currently little information on the retention of carotenoids during the processing of yellow varieties into products such as fermented dried flour and dough [20, 21]. Accordingly, our study focused on the retention of β-carotene in fermented dried flour and dough from yellow cassava varieties using different drying methods. We evaluated the effects of three drying methods on carotenoid depletion during the processing of four varieties of yellow-fleshed cassava into fermented dried flour and its dough.

METHODS

Harvesting and Processing

Tropical *Manihot* Selections of cassava varieties; TMS 0593, TMS 0539, NR 0220 and TMS 1371 were planted earlier during the 2014/2015 planting season at the research farm of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The varieties were planted in the rainy season (May/June) in a replicated randomized complete block design and grown under rain-fed conditions. There was hand-weeding as necessary. There were no fertilizer or herbicides used.

Mature roots (11-12 months old) were harvested, peeled, washed, and soaked for fermentation before processing into flour and dough using sun, oven, and flash drying methods. The roots were from the same harvest batch. We selected small, medium, and large roots across all varieties in a uniform manner. Their weight ranged between 5kg to 7.5kg. We also sorted to remove damaged roots. Precautions were taken to avoid exposure to sunlight during processing to avoid carotenoid degradation

Experimental processing conditions

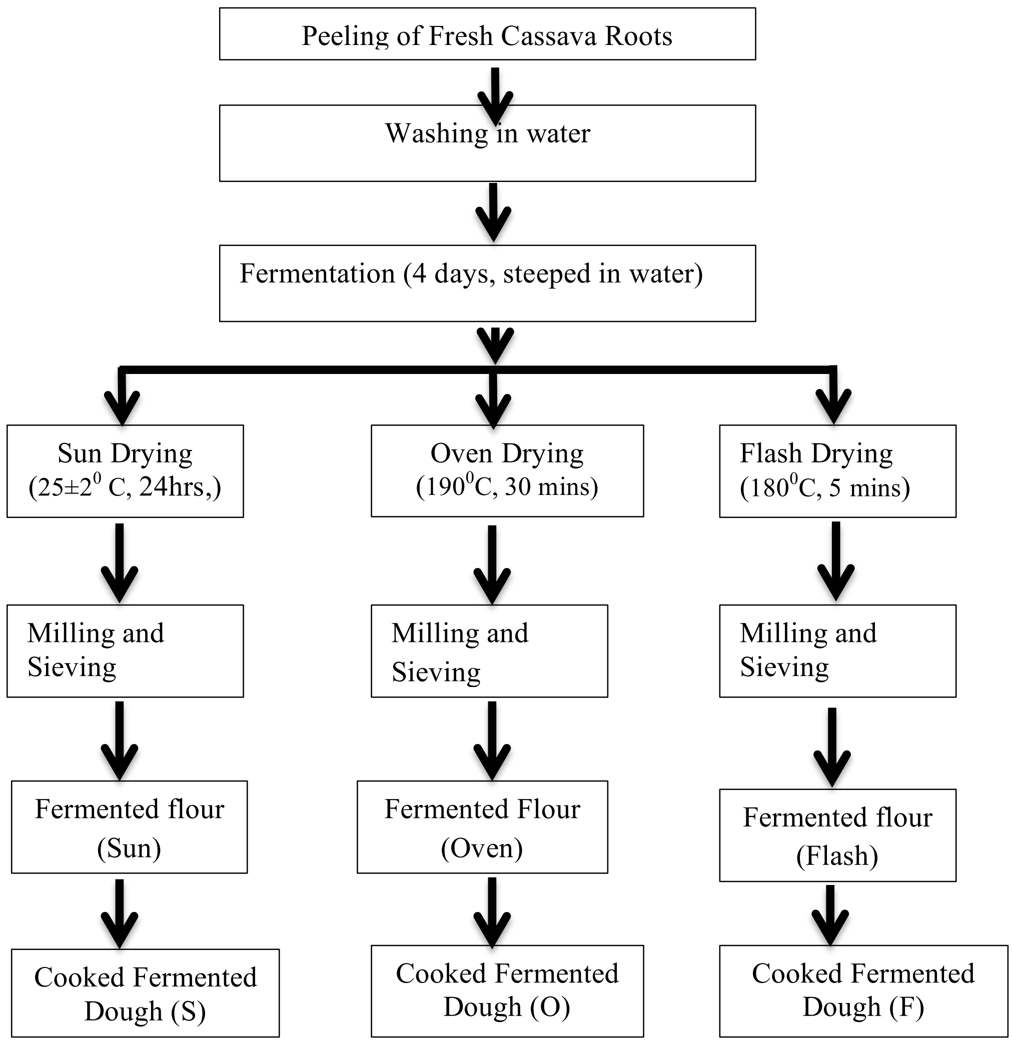
The washed roots were fermented for 4 days. Then we divided the roots into three separate portions for drying using three methods. Sun drying was carried out by spreading the fermented roots of each variety on black polyethylene sheets to avoid contamination; drying took place in 24 hours under direct sunlight during the day and the open air at night.

Temperature observed was between 25±2^0 C while relative humidity was between 30-40% during the period of drying. Oven drying was achieved by placing the fermented root chips in the oven (Gallenkamp, Model QV-440 UK). Drying was carried out at 190^{0}C for 30 minutes.

We conducted flash drying by placing the chipped roots into a locally fabricated flash dryer (with 500kg/day drying capacity). After the roots passed through the drying process at 180^{0}C for 5 minutes, we obtained dried flour. The dried chips from the three drying processes were then milled into flour using a Blender (Waring Blender Model 38BL54, USA).

The flour was introduced into boiling water and stirred vigorously until smooth dough was obtained (Figure 1). The experimental methods followed procedures described [7, 22] for the soaking time; [12, 16, 17] for dewatering, drying, and cooking methods. During processing, we sampled each step three times. The carotenoid analysis was carried out similarly. The results are a mean of analytical values with standard deviation.

Figure 1. Schematic diagram of steps involved in processing of fresh raw cassava roots into fermented flour and corresponding dough.



Extraction of Carotenoids and HPLC Analysis for β-carotene

The extraction from the food products was carried out using methods described by Awoyale et al. [23], with slight modifications in the sample weight to account for changes in the nature of samples at each processing stage. A Waters HPLC machine (Water Corporation, Milford, MA) was used to quantify the isomer forms of β-carotene (*13-cis, 9-cis and trans isomers*).

We used the methods described by Alamu et al. [25] for instrumentation. The methods used by Carvalho et al. [24] and Awoyale et al. [23] were adapted for extrapolation of β-carotene concentrations from chromatograms generated by the HPLC system.

Moisture Content determination

We determined moisture content (MC) using the hot air oven drying method. Samples (fresh roots, intermediate or final products) were oven dried for 24 hours at 105^0C until a constant weight was achieved. Weight was measured before and after drying and used to calculate the weight loss and dry matter content [26].

True Retention Calculation

The formula created by Murphy, Criner, and Gray [27] was used to calculate percentage true retention. The precise weight and moisture content was accounted for at each stage of processing.

$$\frac{\mathbf{Cf \times Wf}}{\mathbf{C1 \times W1}} \; \boldsymbol{X} \quad \mathbf{100}$$

Cf = β-carotene content per gram of processed food
Wf = Weight of processed food
C1= β-carotene content per gram of fresh roots
W1 = Weight of fresh roots before processing

Statistical Analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS) version 20 (IBM *SPSS* Incorporated, Chicago, IL, USA). We obtained mean of triplicate values, Analysis of Variance (ANOVA), and separation of means to test interactions between experimental factors from software.

When testing for interactive effects between methods of processing and type of variety, we set statistical significance at $p<0.05$ for retention values and at $p<0.001$.

RESULTS

The results are presented in Tables 1-3. The tables show β-carotene (µg/g) concentrations and percentage true retention of fermented dried chips, fermented dried flour and dough from fresh roots using the sun drying, oven and flash drying methods respectively. The concentration of β-Carotene in the fresh roots ranged from 5.32 - 6.96 µg/g.

The mean concentration in sun-dried chips was reduced to 6.69 µg/g from 6.71 µg/g, with the percentage true retention ranging from 5.80% in variety 0539 to 26.3% in varieties 1371, with lower mean concentrations in the milled flour (2.17 µg/g) and dough (1.67 µg/g). This was the same for the percentage true retention values at 11.7% and 5.48% respectively.

Table 1. β-Carotene (µg/g) concentrations and percentage (%) true retention of products from Sun drying of yellow Cassava varieties[1]

Varieties	Raw	SD-C	SD-C (%)	SD-MF	SD-MF (%)	SD-D	SD-D (%)
0593	6.75±0.07^{b}	1.63±0.03^{a}	8.50±0.71^{b}	0.50±0.28^{a}	5.70±0.28^{a}	0.08± 0.00^{a}	4.20±0.00^{b}
0539	6.96±0.06^{b}	5.82±0.0^{b}	5.80±0.00^{a}	0.79±0.01^{a}	4.30±1.41^{a}	0.79± 0.00^{b}	3.20±0.00^{a}
0220	7.81±0.13^{c}	9.27±0.04^{c}	14.1±0.17^{c}	3.32±0.00^{b}	10.9±0.00^{b}	3.19± 0.01^{d}	9.30±0.28^{d}
1371	5.32±0.03^{a}	10.03±0.0^{d}	26.3±0.00^{d}	4.08±0.00^{c}	25.7±0.00^{c}	2.62± 0.06^{c}	5.20±0.28^{c}
Mean	6.71±0.96	6.69±3.55	13.7±8.42	2.17±1.67	11.7±9.07	1.67± 1.36	5.48±2.48
S.E	0.52	1.92	4.55	0.90	4.89	0.74	1.34
C.V(%)	15.4	57.4	66.5	82.6	84.0	88.2	48.9

SD-C – Sun Dried Chips SD-MF – Milled flour SD-D – Cooked dough

[1] Mean of triplicate values Means with different letters along columns are significantly different at P< 0.05

Table 2. β-Carotene (μg/g) concentrations and percentage (%) true retention of products from Oven drying of yellow Cassava varieties[1]

Varieties	Raw	OD-C	OD-C (%)	OD-MF	OD-MF (%)	OD-D	OD-D (%)
0593	6.75±0.07[b]	1.44±0.00[a]	5.3±0.00[a]	0.40±0.0[a]	5.1±0.0[a]	0.04±0.00[a]	3.60±0.00[c]
0539	6.96±0.06[b]	3.63±0.00[b]	10.4±0.00[b]	3.41±0.0[b]	4.4±0.0[a]	0.60±0.00[b]	0.98±0.00[a]
0220	7.81±0.13[c]	15.5±0.42[c]	12.2±0.28[c]	11.57±0.4[d]	8.9±1.27[b]	1.78±0.14[c]	6.70±0.28[d]
1371	5.32±0.03[a]	17.4±0.47[d]	16.8±0.57[d]	4.35±0.35[c]	10.8±1.41[b]	1.45±0.21[c]	2.60±0.57[b]
Mean	6.71±0.96	9.49±7.52	11.3±4.45	4.98±4.44	7.30±2.92	0.97±0.74	3.47±0.57
S.E	0.52	4.06	2.38	2.37	1.53	0.40	1.20
C.V(%)	15.4	85.6	42.5	95.9	41.9	81.9	69.4

OD-C – Oven Dried Chips OD-MF – Milled flour OD-D – Cooked dough

[1] Mean of triplicate values Means with different letters along columns are significantly different at P< 0.05

Table 3. β-Carotene (µg/g) concentrations and percentage (%) true retention of products from Flash drying of yellow Cassava varieties[1]

Varieties	Raw	FD-C	FD-C (%)	FD-MF	FD-MF (%)	FD-D	FD-D (%)
0593	6.75±0.07 b	8.84±0.06 d	10.0±2.83 b	4.19±0.01 c	3.40±1.41 c	1.59± 0.14a	0.55±0.08b
0539	6.96±0.06 b	3.84±0.05 b	3.50±0.71 a	2.05±0.07 b	0.60±0.14 a	1.03± 0.03a	0.51±0.01a
0220	7.81±0.13 c	2.30±0.00 a	7.40±0.00 ab	3.53±0.00 d	2.10±0.00 ab	2.13± 0.00b	0.99±0.00c
1371	5.32±0.03 a	6.12±0.00 c	14.6±0.00 c	7.58±0.00 d	7.20±0.00 d	2.74± 0.00c	2.90±0.00d
Mean	6.71±0.96	5.28±2.64	8.88±4.45	3.73±2.67	3.33±2.67	2.48± 0.79	1.24±1.01
S.E	0.52	1.42	2.33	1.44	1.41	0.42	0.56
C.V(%)	15.4	53.9	52.5	77.2	84.9	34.2	91.3

FD-C – Flash Dried Chips FD-MF – Milled flour FD-D – Cooked dough

[1] Mean of triplicate values Means with different letters along columns are significantly different at P< 0.05

The mean concentration of the oven dried chips was 9.49 µg/g with the percentage true retention (%TR) ranging from 5.30% to 16.8%. Mean concentrations were 4.98 µg/g and 0.97 µg/g in milled flour and dough respectively. The mean percentage retention of the oven dried chips were at 7.30% and 3.47% respectively. Among the chips, variety 0220 had the highest β-Carotene concentration and variety 1371 had the highest β-Carotene retention. Variety 0593 had the lowest β-Carotene concentrations among the milled flours and cooked doughs. The mean concentration of the flash dried chips was 5.28 µg/g with the percentage true retention ranging from 3.50% in variety 0539 to 14.6% in variety 1371. Mean concentrations of 3.73 µg/g in the milled flour and 2.48 µg/g in the dough. Mean percentage retention values were 3.33% and 1.24% respectively.

Considering the chips produced using the flash dryer, variety 0593 had the highest concentration of 8.84 µg/g while variety 1371 had the highest true retention of 14.6%. At the milling stage, the flour from variety 1371 had the highest concentration and retention. The retention of β-Carotene in the cooked dough ranged from 0.51% in variety 0539 to 2.90% in variety 1371. Dough produced from oven drying method had a higher mean %TR than dough produced from flash drying. However the reverse was observed for the mean β-carotene concentration of the two methods. A statistical interactive effect was observed at $p < 0.001$ between varieties and methods for all the stages of processing considered in the study.

DISCUSSION

With all three methods, there was a major decrease in the percentage of true retention (%TR) after the drying, suggesting that combinations of exposure to air and heat will severely affect the β-carotene retention. Even though concentration levels were higher in some varieties than in the fresh roots, the increase may be the result of the reduction of moisture and solid losses that increase the carotene per unit weight of the intermediate products. This observation is consistent with other literature [21, 28], where processing resulted in higher carotenoid content per unit weight.

Sun drying had the highest mean %TR in the dried chips when compared with results from the other methods used in this study. Chavez et al. [20] reported that oven drying of cassava chips retained highest carotenoids better than shade and sun drying. On the other hand, Maziya-Dixon et al. [21] reported that sun drying retained carotenoids better than oven drying. These results suggest different methods and duration used in drying in addition to the drying medium could have an impact on retention. The difference may be due to the processing methods since Chavez et al. [20] dried for 2-3 days in the sun, and dried for 6-7 days under a shade.

The higher retention in sun-dried chips is advantageous through its processing into a cooked dough. The higher retention may be partially due to the drying period of one day and the use of black polyethylene sheets, which can reduce drying time compared to drying without the use of sheets [13]. Sun drying is the cheapest and most accessible means of food preservation in developing countries. However, prolonged exposure to the sun can seriously deplete carotenoids in cassava [14, 29]. This is the case in local areas with limited technology, where drying may last for a week, depending on the weather. A reduction in exposure to the sun may reduce and control this depletion. However, drying in less time as achieved in this study is entirely dependent on favorable weather which is defined by low humidity, windy conditions, and hot temperature [30].

The results of oven drying in this study is presented in Table 2. A comparison with previous reports on drying of yellow Cassava shows that Chavez and colleagues oven dried at 60 ^{0}C for a day [20], while Maziya-Dixon et al. [21] dried at 45^{0}C for 72 hours in the production of Cassava chips. However, in this study we conducted drying at 190^{0}C for 30 minutes, which had been pretested before the commencement of the study as the shortest possible time for drying in the oven. The retention values obtained suggest that longer time in the oven, even at lower temperatures, does not help retain β-carotene, as suggested by Onyenwoke et al. [11]. From other studies, we see how lower temperatures to prevent adverse depletion of Carotenoids would take hours and even days for complete drying. As this study was more concerned about practical applications (i.e. industrial Agro-processing) we chose the shortest drying time possible with the selected methods. After considering the cost of power for drying for days in an oven, we chose the high temperature, short drying time method which seemed more adaptable for a realistic setting of large-scale processing. The advantage of flash drying in comparison with other methods is the shorter time of residence, with less than 10 minutes drying time. However, the mean concentrations and retention of flash-dried chips in this study were the lowest. These results suggest the drying medium had a negative impact on the composition of the dried material and therefore may not be suitable for producing fermented dried flour and dough from yellow cassava.

We observed a slight decrease in concentration and subsequent reduction in retention during processing from chips to milled flour in all drying methods, which may be the result of the heat generated in the milling process. Maize processing has similar results, as noted by Suri and Tanumihardjo [31]. Cooking flour into dough had varying impact on the concentrations and percentage of true retention of β-Carotene. While there was a slight decrease for some varieties, other varieties lost considerable amounts of β-carotene and had very low retention. Even though drying time was shortest in the flash drying method, it did not provide substantial retention of β-carotene in the dough compared with dough from sun drying. These results may be due to the higher drying temperature which flash drying involves. The varying impact on concentrations and the percentage of true retention of β-Carotene may also be caused by the difference in cooking time required for each batch. The cooking time varies, as the cooking process requires continuous stirring until a constant dough is achieved. The constant statistical differences support earlier observations that no varieties behave uniformly when the processing method is changed [28, 32]. Therefore, the retention of β- carotene in yellow fleshed cassava as it is processed into fermented dried flour and dough is dependent on variety and the method of processing.

CONCLUSION

The study implies that while varietal-specific effects were evident with different processing methods, varieties with the highest carotene concentration in the fresh root did not necessarily have the highest concentration and retention after processing. Additionally, the results demonstrate that drying provides the desired effect of reducing moisture content but also significantly reduces concentration and retention of β- carotene in the products. This significant loss may become marginal if varieties have higher carotenoid contents in fresh roots, as expected from the ongoing breeding for varieties with higher carotenoid content.

We also observed in our study that the short duration of exposure to the sun during drying, as achieved during this study, may have improved retention of β- carotene. Studies on seasonal and weather changes may be required to determine this advantage. In light of the depleted levels of β- carotene in the final dough from all three methods, processing technologies that could minimize β- carotene loss should be investigated. The values obtained for final consumable products in this study are lower than expected of a product intended for improving vitamin A intake.

Nevertheless, consumption of this product with vegetables and stews could complement the total supply of dietary provitamin A. While vegetable consumption is not a substitution for this loss, diversity in diets with vegetables may improve total vitamin A intake. The results of β-carotene depletion presented in this study will assist ongoing plant breeding in the selection of varieties and biofortification efforts aimed at increasing pro-vitamin A content in Cassava.

Competing Interests: The authors declare no conflict of interest.

Authors' Contributions: TE, BMD, RS designed the research. TE and BMD performed the experiment. TE, RS, BMD, and EA prepared the manuscript.

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